Strenuous exercise simulating hepatic injury during vaccine trials

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Three healthy young men participating in phase 1 clinical vaccine trials had unexplained increases in their serum transaminase levels. Retrospective analysis indicated that these volunteers had participated in strenuous physical training 2–5 days prior to the noted elevations. The pattern of serum enzyme elevations, initially thought to be consistent with hepatic injury, were associated with parallel increases in creatine phosphokinase. One individual consented to repeat his exercise regimen. This was followed by a recurrence of the same pattern of increases in serum enzymes, including creatine phosphokinase. Thus, in trials where serum enzymes will be measured, it may be prudent to encourage subjects to refrain from increasing their activity above that which they normally perform.

Keywords: Exerise; creatine phosphokinase; hepatic injury; vaccine trials; transaminase

INTRODUCTION

Serum enzyme abnormalities that occur after muscle injury have been described repeatedly in studies concerned with pigmenturia and acute renal damage associated with exercise[1–3]. For exercises as brief as 6 min, serum creatine phosphokinase (CK) can rise significantly in the untrained individual, whereas trained individuals have lesser increases[4]. Physical exertion at a level associated with military training can result in a syndrome known as acute exertional rhabdomyolysis, in which significant elevations of serum lactate dehydrogenase (LDH), aspartate aminotransferase (AST, SGOT) and CK accompany elevations in serum myoglobin[5].

In recent phase 1 clinical studies, we had cause to examine the serum enzyme changes characteristic of acute exertional rhabdomyolysis. Three individuals participating in three distinct vaccine trials experienced transient serum enzyme abnormalities. This report describes the circumstances associated with those elevations and explains those changes based on associated laboratory abnormalities most consistent with acute exertional rhabdomyolysis.

MATERIALS AND METHODS

Volunteers

Volunteers participating in clinical studies of live, attenuated-virus vaccine candidates for chikungunya and dengue fever and one inactivated-virus vaccine candidate for hepatitis A were among the active-duty soldiers stationed at Fort Detrick, Frederick, MD. Duty assignments were in laboratory settings where physical exertion was not required and physical training beyond that required of such active-duty soldiers was not critical. Soldiers were expected to pass physical training standards (including height, weight, sit-ups, push-ups and a 2 mile run) twice a year, but were not involved in scheduled training between tests.

Prior to enrolment in clinical studies, volunteers underwent complete history, physical examination and laboratory screening, including measurement of serum chemistry parameters. Enzyme measurements were included in a liver profile as a standard part of that screening, whereas serum creatine kinase (CK) levels were not included.

Typically, volunteers were part of either out-patient or in-patient studies, based on the degree and type of monitoring required. For in-patient trials, subjects were admitted to a closed clinical research ward for 3 days before administration of the trial vaccine or product. This period allowed for the expression of incubating acute upper-respiratory illnesses. During the following 1–2 weeks, volunteers were confined to the clinical research ward where they were closely monitored. During that time the volunteers were allowed to participate in minor sports such as table tennis and billiards or exercise in a complete fitness room, which included free weights, resistance weight machines, a rowing machine, and a stationary bicyle. Subjects participating in out-patient studies were seen periodically for evaluation and for blood sampling, depending on the nature of the study.

Clinical laboratory assays

Serum samples were analysed in a College of American Pathology-approved clinical laboratory, USAMRIID, using an Abbott Spectrum Chemistry Analyzer (Abbott Laboratories, Irving, TX) with reagents, rinsing solutions and standards manufactured by Abbott Laboratories. Expected normal ranges for assays were as follows: alkaline phosphatase (AP) 36–92 IU l^{-1}; aspartate aminotransferase (AST, SGOT) 16–40 IU l^{-1}, alanine

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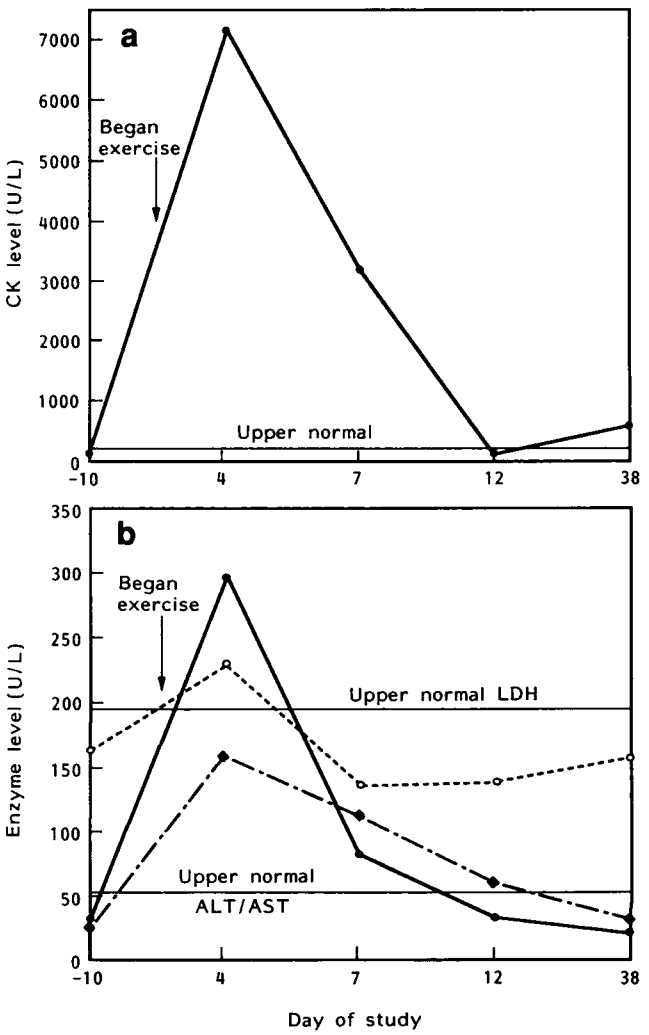

Figure 1 Serum creatine kinase (a) and AST, ALT, and LDH (b) levels for case no. 1. Enzyme levels in serum samples from the days indicated were determined as described in Materials and methods. Solid lines indicate upper limits of normal laboratory values for LDH, AST and ALT. Values for AST (——●——), ALT (——◆——) and LDH (- - -○- - -) for the days indicated

aminotransferase (ALT, SGPT) 8–54 IU l^{-1}, lactate dehydrogenase (LDH) 109–193 IU l^{-1}, total bilirubin 0.4–1.1 mg/dl^{-1}, gamma glutamyl transferase (GGT) 11–63 IU l^{-1} and creatine kinase (CK) 45–235 IU l^{-1}.

RESULTS

Case no. 1

This subject was a healthy 33-year-old Caucasian male who participated in a live, attenuated chikungunya virus vaccine in-patient trial in which he responded serologically to the vaccine; his only symptoms were myalgia. This subject received a single dose of vaccine subcutaneously on day 0. Physical examinations during the trial were unremarkable. Serum samples for day 4 of the trial showed notable elevations in AST and ALT with normal bilirubin, AP and LDH.

Upon questioning, the subject related his myalgia to strenuous physical activity which he began on day 1 of the vaccine trial and terminated, at our request, on day 5. The subject was attempting to lose weight for an upcoming physical training test. Based on these findings, CK and GGT levels were determined on available serum samples. Serum GGT levels were normal. However, as seen in *Figure 1a*, CK levels were markedly elevated on day 4 of the study and returned to baseline by day 12. This rise and fall of CK paralleled the changes in AST and ALT seen in this subject (*Figure 1b*). Of interest is that, although LDH levels did not rise significantly above normal, there was a slight increase in LDH on day 4.

Case no. 2

This subject was a healthy 20-year-old Hispanic male who participated in a live, attenuated dengue 4 virus vaccine in-patient trial in which he failed to respond serologically to the vaccine; he reported no symptoms during the trial. This subject received a single dose of vaccine subcutaneously on day 0. Physical findings during the trial were unremarkable. Laboratory studies on day 4 of the trial showed notable elevations in AST and LDH with slightly elevated ALT and normal bilirubin and AP.

Upon questioning, this subject related that he had begun weight training beyond his usual level of activity on day −2 of the vaccine trial. He terminated this activity on day 5. Based on these findings, CK and GGT levels were determined on available serum samples. Serum GGT levels were measured for day 0, 1 and 5 and were normal. However, as seen in *Figure 2a*, CK levels were markedly elevated on day 5 of the study and returned to baseline by day 12. We did not have serum samples to determine the CK levels between day 1 and day 5; thus a full temporal description of the relationship between the CK elevation and changes in AST and LDH seen in this subject (*Figure 2b*) could not be determined.

Case no. 3

This healthy 23-year-old Caucasian male participated in an inactivated hepatitis A virus vaccine out-patient trial in which he did not seroconvert to hepatitis A. This subject received a single dose of vaccine subcutaneously on day 0 and a second dose on day 30, at the time of blood sampling. On day 30, examination of the previous vaccination site was unremarkable and the subject reported no symptoms. Laboratory studies of serum drawn on day 30 prior to inoculation showed significant elevations in AST with normal bilirubin, ALT, AP and LDH levels.

Upon questioning, this subject related that he had increased his physical training regimen significantly during the week prior to the blood drawing and administration of the second dose of vaccine. He denied the use of alcoholic beverages. The subject related that he had diffuse myalgia, which he attributed to the increased activity, and had terminated his activity because of the myalgia. Based on these findings, CK and GGT levels were determined on available serum samples. Serum GGT levels were normal. However, as seen in *Figure 3a*, CK levels were markedly elevated on days 30 through 34 of the study and returned to baseline by day 36. This rise and fall of CK paralleled the changes in AST, ALT and LDH seen in this subject (*Figure 3b*). In this case, the peak enzyme levels occurred ≈5–7 days after the subject increased his activity and resolved ≈9–11 days later.

This subject agreed to resume his increased physical training regimen once his enzyme levels returned to

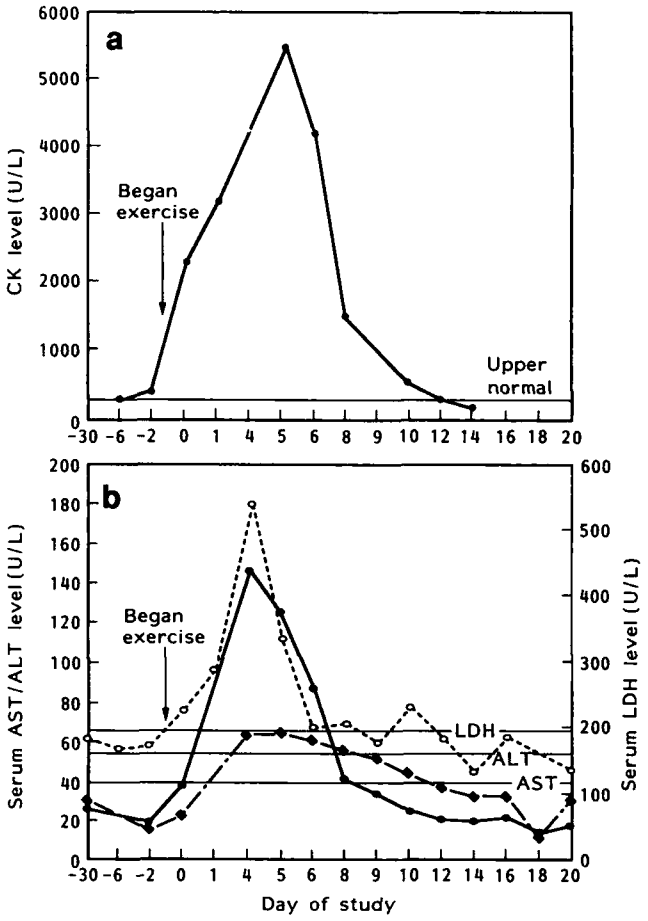

Figure 2 Serum creatine kinase (a) and AST, ALT, and LDH (b) levels for case no 2. Enzyme levels in serum samples from the days indicated were determined as described in Materials and methods. Solid lines indicate upper limits of normal laboratory values for LDH, AST and ALT. Values for AST (——●——), ALT (——◆——) and LDH (- - -○- - -) for the days indicated

normal (day 38). As noted in *Figure 3*, a pattern of increased CK, AST and LDH peaked 4 days after he resumed exercise and paralleled the increases seen earlier in this same subject. Of interest is that these changes were not as severe as in the first episode and the subject reported less severe myalgia. There was no way to evaluate quantitatively his level of activity in the two sessions. Thus, it is unclear whether the smaller increase in CK levels was a result of conditioning associated with the first episode or because he did not exercise as strenuously the second time.

DISCUSSION

In each of the three cases presented, there was initial concern that there was subclinical hepatic injury caused by the vaccines. However, there was no clear association of the elevated AST, ALT and LDH levels with any one particular aspect of the clinical trials. Of the two volunteers who received live vaccines, only one, as evidenced by seroconversion, was infected with the vaccine virus. Neither of the volunteers had a pattern of symptoms associated with the natural disease for which the vaccines were developed, or had fever or objective evidence of infection such as rash, leukopenia or lymphocytosis.

The subject who received the inactivated vaccine had normal blood chemistries 2 weeks after the first inoculation (data not shown). Athough clinical hepatitis following an inactivated hepatitis A vaccine would be of particular concern because of the suggestion of incomplete inactivation, there was no other objective evidence of hepatitis, including hepatitis serology and stool cultures (not shown). In addition, other preclinical and clinical data indicated that it is highly unlikely that there was any residual live virus in this vaccine (Sjogren, personal communication).

After reviewing these cases, a common factor emerged: these volunteers had been relatively sedentary until 2 to 7 days before the enzyme elevations, at which time they had begun a strenuous exercise regimen in preparation for physical fitness tests. Two of the three had significant myalgia associated with that activity. Taken together, the data indicate that these volunteers were physically untrained and abruptly began vigorous physical activity. The enzyme elevations noted are consistent with the observations by others in which acute increases (within 15 min) in serum AST, ALT, LDH and aldolase levels are greatest in untrained individuals after a single exercise

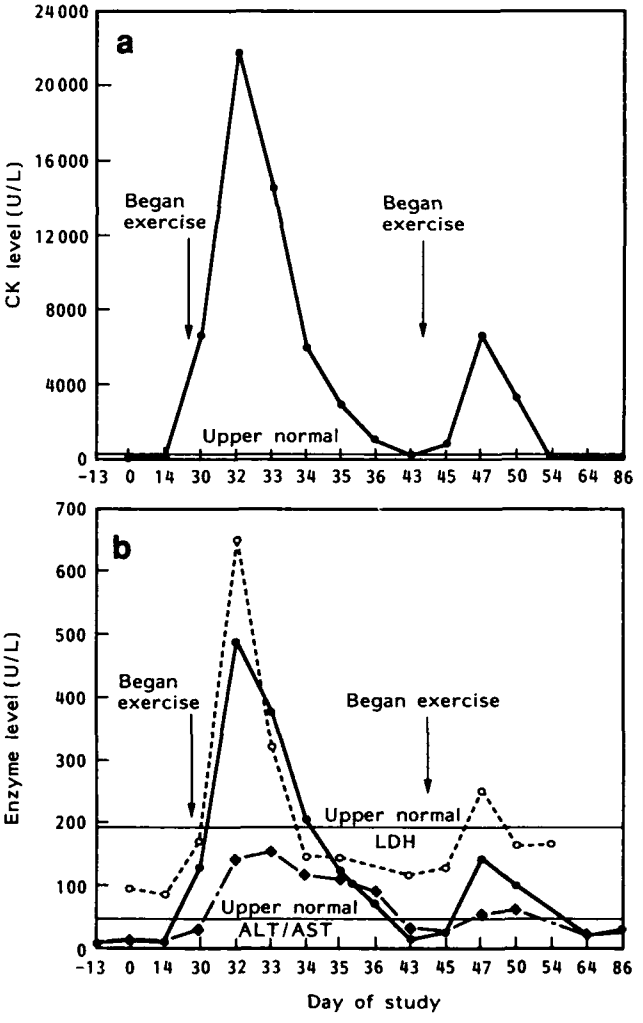

Figure 3 Serum creatine kinase (a) and AST, ALT, and LDH (b) levels for case no. 3. Enzyme levels in serum samples from the days indicated were determined as described in Materials and methods. Solid lines indicate upper limits of normal laboratory values for LDH, AST and ALT. Values for AST (——●——), ALT (——◆——) and LDH (- - -○- - -) for the days indicated

session[6]. Notably, the elevations seen in the three cases presented here were much beyond the acute changes noted[6,7]. However, reports show peaks in CK levels in unconditioned athletes at 10–20 h after a single episode of moderate exercise[4], at 48 h with eccentric muscle exercise[8], 3–4 days after recreational exercise[9] and up to 7 days after completion of a marathon race[10]. In our cases, the volunteers were not subject to one single exercise period; rather, they exercised on successive days. Therefore it is not unlikely that the peaks in CK, AST, ALT and LDH levels might be several days later and, depending on clearance of the enzymes, might accumulate to higher levels. Indeed, Olerud *et al.*[11] reported that marine recruits who initiated physical exercise during basic training had peaks of serum CPK, AST, LDH and myoglobin during the first 6 days of regular training.

The fact that the pattern of enzyme elevations was reproduced in the third subject when he repeated his increased activity supports the contention that the enzyme elevations were a result of increased physical activity rather than a side-effect of vaccination. Furthermore, the absence of other indications of hepatocellular damage (e.g. normal GGT, bilirubin) suggests that the abnormalities seen were not the result of hepatic injury. It is also unlikely that the enzyme elevations were the result of cardiac damage as the pattern of elevations was not consistent with cardiac injury, the CK MB fractions were normal (not shown), and the subjects had no cardiac symptoms or history to suggest coronary artery disease.

Unfortunately, there is no single uniformly specific routine serum chemistry test that is useful in distinguishing between muscle and liver damage. AST, ALT and LDH are released by muscle as well as liver after injury. GGT is relatively specific for liver function, especially that associated with hepatotoxic drugs such as ethanol or phenobarbital. Specific tests of serum myoglobin have been reported as a reliable marker of muscle tissue damage[11]: however, assays for this are best done on unfrozen specimens and are not uniformly available. Thus, a uniform and convenient test to differentiate liver dysfunction from muscle tissue abnormalities remains to be developed. Additional prospective studies of the association of elevations of serum enzyme levels with exercise in trained and untrained volunteers are warranted.

The management of clinical trials is complex and distinguishing between causal relationships and temporal associations is often difficult. Thus, limiting volunteer selection and using matched controls, placebo controls and blinding in prospective trials have proven useful. Nevertheless, there are those occasions for which retrospective analysis provides data useful in explaining unusual observations. In the cases presented here, physical activity may have interfered with acquiring reliable data concerning the vaccines under study. Thus, in trials where serum enzymes will be measured, it may be prudent to encourage subjects to refrain from increasing their activity above that which they normally perform.

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